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NADPH

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Abbreviations:
glutathione peroxidase:GPx
$NADP^+$—nicotinamide adenine dinuclotide phosphate
NADPH— nicotinamide adenine dinuclotide phosphate reduced form
glutathione reductase: GR
reduced glutathione GSH
oxidized glutathione disulfide GSSG
superoxide dismutase (SOD)
ROS—reactive oxygen species

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Abstract:

Nicotinamide adenine dinuclotide phosphate reduced form (NADPH), an electron carrier, is a kind of antioxidants against the oxidative stress in the mitochondria. The major resources of NADPH are the pentose phosphate pathway and photosynthesis. This paper will focus on the structure, formation, reaction, detection and stability of NADPH.

Introduction:

Recently, reactive oxygen species (ROS), which are generated by oxidative stress, are considered as cytotoxic products of cellular metabolism and play an important role in the growth, division, transformation, and apoptosis in mammalian cells. ROS has been involved in many human diseases, such as aging, diabetes, stroke and many types of cancers. The damages induced by ROS attack take place in mitochondria that has been demonstrated as one of the major sources of ROS. ROS can directly damage mitochondrial enzymes [1], and cause mitochondrial DNA mutation [2]. In the process of these damages in mitochondria, superoxide anions ($O_2^{\bullet -}$) are produced by the leakage of electrons, and then rapidly reduced to H_2O_2 by manganese superoxide dismutase (SOD). H_2O_2 can be converted to H_2O by the function of catalase. However, catalase is absent in the mitochondria of most animal cells, mitochondrial glutathione peroxidase (GPx) plays a key role in metabolizing H_2O_2. Reduced glutathione (GSH) known as an efficient radical scavenger is very important for the activity of mitochondrial GPx. GSH is known to be synthesized in the cytosol and transported into the mitochondria [3-4]. GSH converts to oxidized glutathione disulfide (GSSG) in the mitochondria which cannot be exported into the cytosol for reconversion into GSH. Therefore, mitochondrial NADPH becomes a necessary reducing equivalent for the regeneration of GSH from GSSG by the activity of mitochondrial glutathione reductase (GR). Because of the importance of NADPH in reductive system, it becomes one of the best antioxidant available against the oxidative stress in the mitochondria.

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Structure of NADPH

NADPH is a water-soluble two-electron carrier. $NADP^+$ is the oxidized form which lacks electrons. Nicotinamide nucleotide-linke dehydrogenase can catalyze the reversible reaction that $NADP^+$ accept electron and convert to NADPH (reaction 1). Compared to NADH, which

donates electrons to the electron transport system for energy generation, NADPH donates electrons to biosynthetic reactions [5].

$$NADPH + NADH^{+} \leftrightarrow NADPH + NAD^{+} \quad \text{(reaction 1)}$$

In cells, NADPH is produced by the pentose phosphate pathway. In plants, photosynthesis is another source of NADPH.

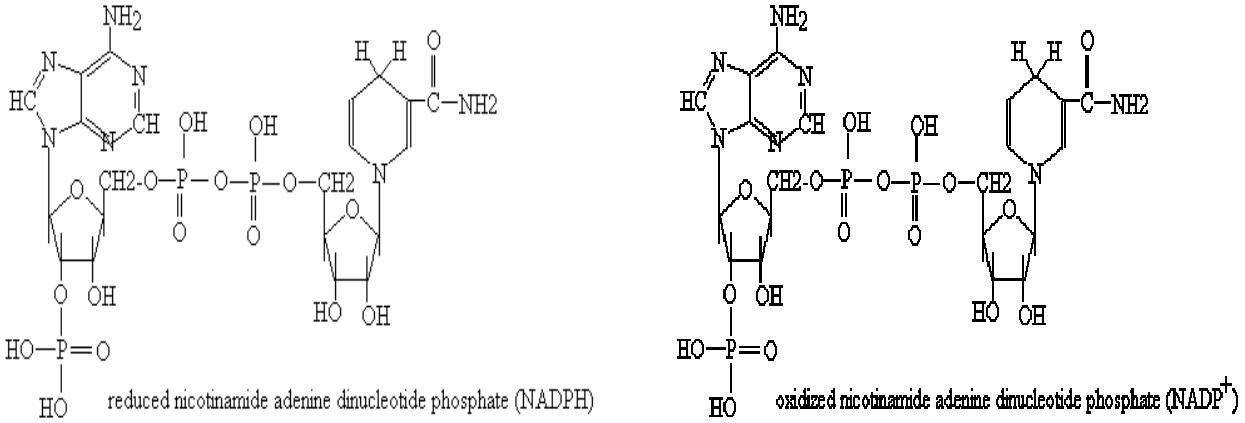

Figure1: The structure of NADPH and $NADP^{+}$ [5] $NADP^{+}$ can accept one electron and convert to NADPH. The reaction mainly takes place in mitochondria.

Two major pathway of NADPH generation

Pentose Phosphate Pathway

In biosynthetic reactions, the pentose phosphate pathway is an importance of generating NADPH. It operates exclusively in the cytosol. The pentose phosphate pathway includes oxidative phase which produces NADPH, and non-oxidative phase which produce pentoses and miscellaneous other sugar phosphates [6]. In oxidative phase, there exist four reactions. Glucose-6-phosphate dehydrogenase is the key enzyme that is responsible for the generation of NADPH [7]. Evidence shows that it reduces cellular oxidative stress by increasing the GSH concentration [8].

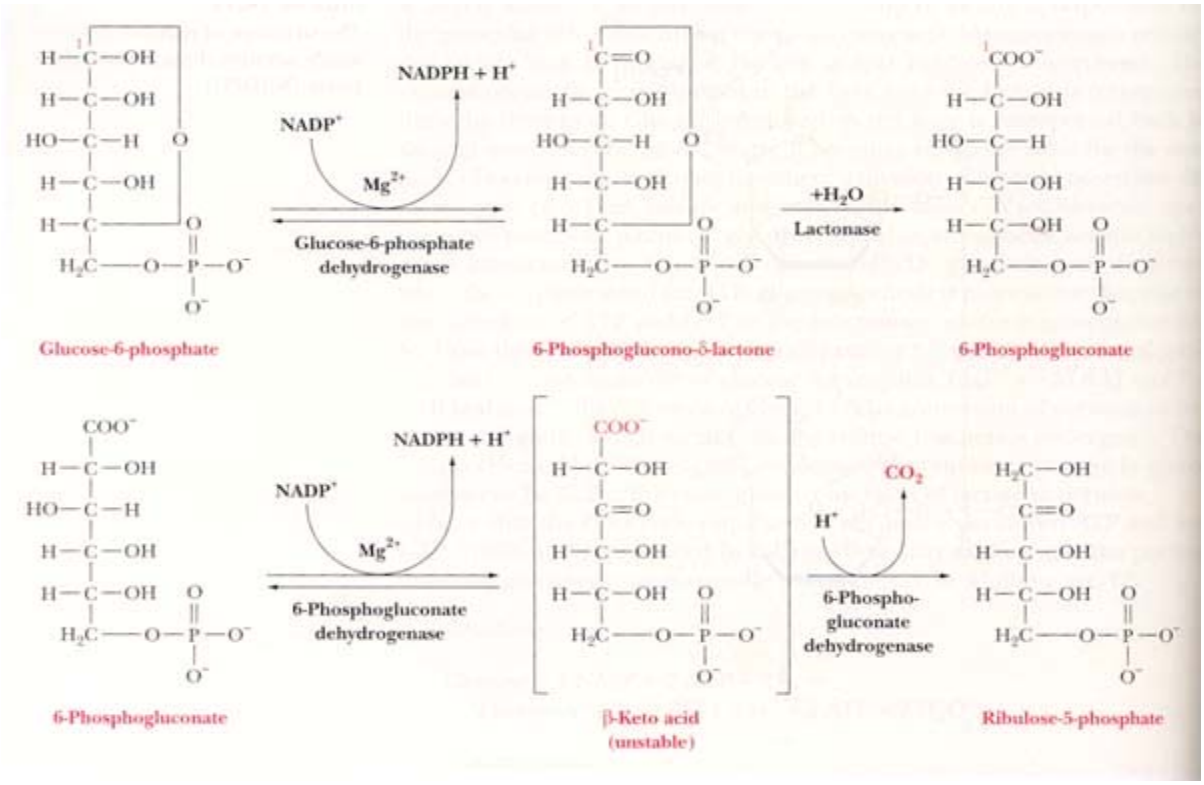

Figure 2: Generates NADPH:the oxidative reaction of the pentose phosphate pathway [9]. In this pathway, the electron acceptor is $NADP^+$ rather than NAD^+, which is reduced to NADPH; the reaction has two irreversible steps. Therefore, the reaction continues until nearly all of the NADP+ has been reduced to NADPH

Photosynthesis pathway:

Photosynthesis pathway encompasses two processes: the light reactions when plants are illuminated, and the carbon-assimilation or carbon-fixations reactions, usually called dark reactions that produce carbohydrate. In eukaryotic cells, both light reactions and dark reactions take place in chloroplast. When cells absorb light, H_2O is converted to O2 and $NADP^+$ is reduced to NADPH coupled the phosphorylatio of ADP to ATP (reaction 2) [9]. In dark reactions, ATP and NADPH are consumed to reduce CO_2 to form sucrose, starch and other derived products (Figure 3).

$$NADP^+ + H^+ + 2e^- \rightarrow NADPH \qquad \Delta E^{\circ\prime} = -0.32V$$

$$1/2\ O_2 + 2H^+ + 2e^- \rightarrow H_2O \qquad \Delta E^{\circ\prime} = -0.816V$$

Net reaction: $NADP^+ + H_2O \rightarrow NADPH + H^+ + 1/2\ O_2$ $\Delta E^{\circ\prime} = -1.136V$ (reaction 2)

$\Delta G^{\circ\prime} = 220 kJmol^{-1}$

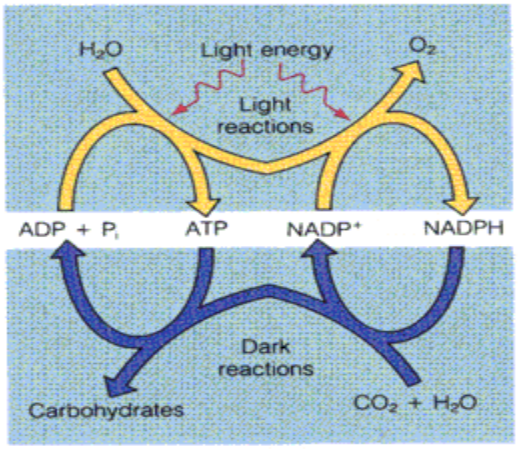

Figure 3: The two subprocesses of photosynthesis: light reactions and dark reactions [3]. Electrons from H_2O to CO_2 and be transferred to $NADP^+$ form NADPH, and releasing O2.

Detection and measurement of NADPH

Usually, the values of molar absorptivities (epsilon) are used as the measurement parameter. Evidence has shown that the maximum of absorbance of NADPH is at the wavelength about 340nm. Only nearly 334 nm, NADPH is almost identical and nearly independent of temperature. Therefore this wavelength is recommended for precise measurements [10]. 260nm is also another parameter for detection and measurement of NADPH, but the 260 nm/340 nm absorbance ratio is about 2.265 [11].

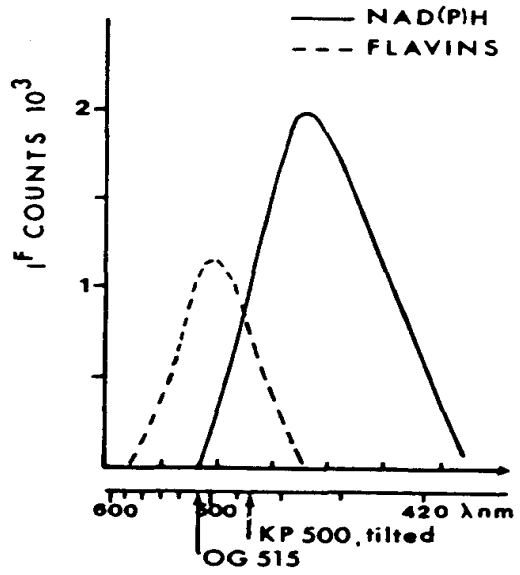

Figure 4: Emission spectra of NADPH and flavins as they have been resolved from actual measurement on single [6].

Stability of NADPH:

Because of the wide application of NADPH, the information on NADPH stability is very important to help decrease the degradation during storage and assay. Evidence shows that many factors can influence the rate of NADPH degradation, such as PH, temperature, ionic strength, phosphate and acetate. Experiments are designed to determine those factors effect on NADPH degradation. The loss of absorbance at 340nm is measured followed first-order kinetics when NADPH degrades. The rate constant of different temperature can be calculated using equation 1 at constant PH. A is the absorbance at 340nm either at time zero or at time t. With the change of PH, the rate constant can be obtained by equation 2. A similar expression can be used in presence of phosphate and acetate (equation 3).

$$k_1=2.303t/\log A_o/At \quad (1)$$

$$\log k_1' = \log k_1 - PH \quad (2)$$

$$\log k_1'' = \log k_1 + \log [\text{phosphate and acetate}] \quad (3)$$

As table 1 shows, PH has the greatest effect on NADPH stability, which suggest that NADPH should be stored at a slightly alkaline condition (PH>8). NADPH is much less stable at high temperature; so it is necessary to be aware of the liability of NADPH at 37°C when test the enzyme activity. Phosphate and acetate also can accelerate the rate of NADPH degradation [12].

Table 1: Summary of the rate of NADPH degradation under various conditions [4]. The first-order rate constant *k* is measured at different conditions. The initial concentration of NADPH is 1×10^{-4}mol/L.

Reaction of NADPH:

NADPH, a coenzyme in redox system, has the ability not only to reduce GSH, but react with many other species, such as enzymes, oxidized protein, metal and amino acids. GSH has been known as an important antioxidant which needs NADPH involving the regeneration from oxidize form GSSH to GSH in mitochondria (reaction 3).

$$GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+ \quad \text{(reaction 3)}$$

The oxidative stress can lead some protein intramolecular SH-groups to S-S crosslinking which causes deleterious conformational changes. Crosslinking of *intermolecular* SH-groups

leads to the deleterious aggregation of several protein molecules (reaction 4).Therefore the cells have developed ways to keep these sulfhydryl groups. NADPH is used to reduce protein sulfhydryl groups that have been spontaneously oxidized by reactive oxygen species (reaction 5). This is especially important in red cells which, as oxygen carriers for the organism, are loaded with oxygen and therefore containa high concentration of reactive oxygen species.

$$\text{protein S-S- protein} + 2H \rightarrow \text{protein-} (SH)_2 \qquad \text{(reaction 4)}$$

$$\text{protein-S-S-protein} + NADPH \leftrightarrow 2\ \text{protein-SH} + NADP^+ \qquad \text{(reaction 5)}$$

NADPH also plays an important role in the pathway of formation of glutamate. Glutamate is the major source of amino groups for most other amino acids through transamination of reactions. The reaction of α-ketoglutarate and $NH4^+$ to form glutamate in one step is catalyzed by L-glutamate dehydrogenase which presents in all organisms. The reducing power is provided by NADPH (reaction 6) [5].

$$\alpha\text{-ketoglutarate} + NH4^+ + NADPH \rightarrow \text{L-glutamate} + NADP^+ + H_2O \qquad \text{(reaction 6)}$$

Summary:

NADPH plays an essential role in decreasing the effect by ROS damage in mitochondria. NADPH is temperature-dependent and PH-dependent, which need careful storage. As an electron carrier, NADPH can reduce GSSH, protein, amino acids and many other molecules. It also has been known as a coenzyme involving many reactions in cells.

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